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# Prebiotic Synthesis of Hydrophobic and Protein Amino Acids

**(electric-discharge synthesis/gas chromatography–mass spectrometry/amino-acid analyzer)**

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**ABSTRACT** **The formation of amino acids by the action of electric discharges on a mixture of methane, nitrogen, and water with traces of ammonia was studied in detail. The presence of glycine, alanine, α-amino-*n*-butyric acid, α-aminoisobutyric acid, valine, norvaline, isovaline, leucine, isoleucine, alloisoleucine, norleucine, proline, aspartic acid, glutamic acid, serine, threonine, allothreonine, α-hydroxy-γ-aminobutyric acid, and α,γ-diaminobutyric acid was confirmed by ion-exchange chromatography and gas chromatography–mass spectrometry. All of the primary α-amino acids found in the Murchison Meteorite have been synthesized by this electric discharge experiment.**

Most prebiotic syntheses that start with the primitive atmospheric constituents give substantial yields of glycine, alanine, and α-amino-*n*-butyric acid (1). Prebiotic syntheses of the higher aliphatic amino acids have been claimed, for example, by the action of electric discharges on $CH_4 + NH_3 + H_2O$ (2–6), by heating $CH_4 + NH_3 + H_2O$ to 900–1200° (7, 8), and by the action of shock waves on $CH_4$, $C_2H_6$, $NH_3$, and $H_2O$ (9). The amino acids were identified only by an amino-acid analyzer (2–4, 6, 7, 9), only by paper electrophoresis (8), or only by gas chromatography (5). However, these techniques are not sufficient by themselves to identify an amino acid.¶

In the original synthesis of amino acids by electric discharges (10–12), only glycine, alanine, α-amino-*n*-butyric acid, α-aminoisobutyric acid, and β-alanine, of the simple aliphatic amino acids, were synthesized in sufficient yield to obtain identification by a melting point of a derivative. Recently developed techniques permit the identification of compounds found in lower yield by this synthesis.

The synthesis under prebiotic conditions of aspartic and glutamic acid (2–8, 12–14), serine (2, 5, 6–8, 13), threonine (2, 3, 5–7), and proline (3, 4, 7, 8, 13) have been reported but they have not been properly identified [except for aspartic acid (14)]. The synthesis of these amino acids (except proline) has also been reported from the polymerization of HCN (1), but again without proper identification. A prebiotic synthesis of threonine should also yield allothreonine, but this amino acid has never been reported. In addition, several investigators have reported the appearance of a large peak at the isoleucine position on the amino-acid analyzer (2, 4–6, 13). The identification of this peak as isoleucine has been questioned (4, 13). It is evident that this compound cannot be isoleucine, since a corresponding peak for alloisoleucine is not observed.

Most electric-discharge experiments have been done with a large amount of ammonia present. Use of nitrogen instead of ammonia in such experiments does not change the major products, although the yield of amino acids is lower (12). The use of a higher concentration of ammonia in such experiments has been criticized (15), and it is now thought that the ammonia concentration in the prebiotic atmosphere was not likely to have been greater than $10^{-5}$ atm (16, 17). Although this is a small percent of the atmosphere, this value corresponds to a significant concentration of $NH_4^+$ and $NH_3$ in the ocean, where $NH_3$ would have played an important role in prebiotic synthesis of organic compounds.

We have synthesized a wide variety of amino acids by the action of electric discharges on a mixture of methane, nitrogen, and water, with traces of ammonia. The compound chromatographing as isoleucine on the amino-acid analyzer is shown to be α-hydroxy-γ-aminobutyric acid. These amino acids were all positively identified by gas chromatography–mass spectrometry.

## MATERIALS AND METHODS

To avoid contamination from reagents, the HCl was distilled, constant boiling. $NH_4OH$ was prepared by adding gaseous $NH_3$ to water redistilled from alkaline permanganate. $NH_4Cl$ was recrystallized from water. Authentic samples of the amino acids were obtained from Calbiochem, except for α-hydroxy-γ-aminobutyric acid, which was prepared by selective deamination of α,γ-diaminobutyric acid (18). *Sec*-Butanol was 90% of the (+) butanol isomer (Norse Laboratories, Santa, Barbara, Calif.).

To a 3-liter flask with two tungsten-electrodes (4) was added 100 ml of 0.05 M $NH_4Cl$. The flask was evacuated, and sufficient $NH_3$ was added to bring the pH to 8.7. Methane (200 mm) and $N_2$ (200 mm) were then added, and the spark discharge was run for 48 hr. The tesla coil was the same kind

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¶ The correct elution time on the amino-acid analyzer is obviously insufficient by itself to identify an amino acid. Many nonprotein amino acids have peaks that coincide with the protein amino acids. This is clearly shown in Hamilton, P. B. (1963) *Anal. Chem.* **35**, 2055–2064. The same limitation is true for paper chromatography or electrophoresis, even with several different solvents. Coincidence of the radioactivity of an unknown with the color of a known sample on paper chromatography has led to several errors. The only methods that seem reliable for amino acids are identification by the melting point and mixed melting point of a suitable derivative, or analysis by gas chromatography–mass spectrometry.

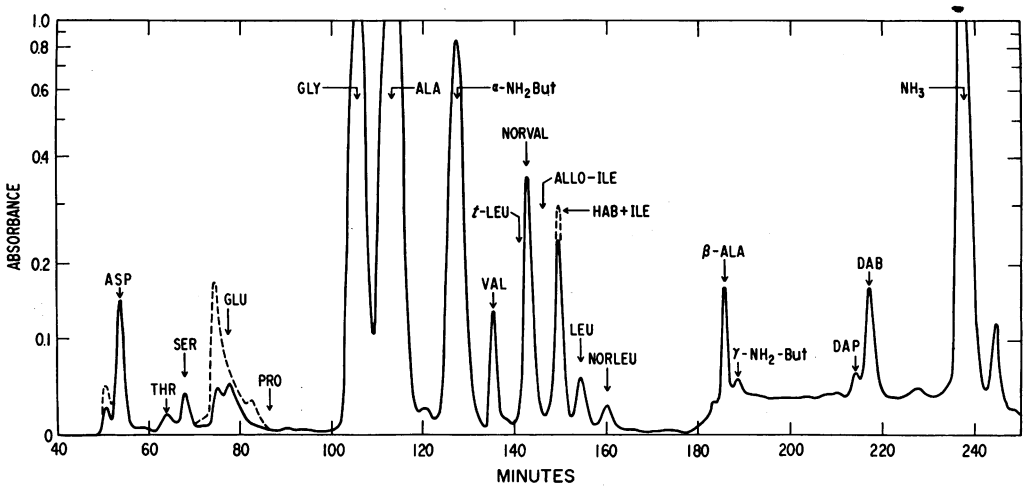


FIG. 1. Chromatogram from an amino-acid analyzer of the desalted amino acids after the electric discharge synthesis. The *arrows* show the elution time of the indicated amino acids. The *dashed line* shows the increase in color on heating the ninhydrin–buffer mixture for 30 min instead of 8 min. (*solid line*). *DAP* is $\alpha,\beta$-diaminopropionic acid. *Norleu* is norleucine; *Norval* is norvaline; *DAB* is $\alpha,\gamma$-diaminobutyric acid; *HAB* is $\alpha$-hydroxy-$\gamma$-aminobutyric acid.

as previously used (11). The temperature of the flask remained between 20° and 25°. The aqueous solution, presumably containing the amino nitriles rather than the amino acids (12), was hydrolyzed with 3 M HCl for 24 hr, desalted, and evaporated to dryness. The dried sample was hydrolyzed again with 3 M HCl in order to open the rings of glutamic acid, $\alpha,\gamma$-diaminobutyric acid, and $\alpha$-hydroxy-$\gamma$-aminobutyric acid that may have been formed during the desalting. Seven similar runs were combined. A sample of the desalted amino acids was then run on the amino-acid analyzer (19).

In order to separate the various amino acids, the combined runs were chromatographed on Dowex 50($H^+$) (38.5 × 2.2 cm) and eluted with HCl (20) (400 ml of 1.5 M HCl, 700 ml of 2.5 M HCl, 400 ml of 4.0 M HCl, and 600 ml of 6.0 M HCl).

TABLE 1. *Yields and mole ratios of amino acids from sparking 336 mmol of $CH_4$*

| | μmol | Mole ratio | | μmol | Mole ratio |
|---|---|---|---|---|---|
| Glycine | 440 | 100 | Norleucine | 6.0 | 1.4 |
| Alanine | 790 | 180 | *tert*-Leucine | <0.02 | — |
| α-Amino-*n*-butyric acid | 270 | 61 | Proline | 1.5 | 0.3 |
| α-Aminoisobutyric acid | ~30 | ~7 | Aspartic acid | 34 | 7.7 |
| Valine | 19.5 | 4.4 | Glutamic acid | 7.7 | 1.7 |
| Norvaline | 61 | 14 | Serine | 5.0 | 1.1 |
| Isovaline | ~5 | ~1 | Threonine | ~0.8 | ~0.2 |
| Leucine | 11.3 | 2.6 | Allothreonine | ~0.8 | ~0.2 |
| Isoleucine | 4.8 | 1.1 | α,γ-Diaminobutyric acid | 33 | 7.6 |
| Alloisoleucine | 5.1 | 1.2 | α-Hydroxy-γ-aminobutyric acid | 74 | 17 |

The yields of glycine and alanine, based on the carbon, are 0.26% and 0.71%, respectively. The total yield of amino acids listed in the table is 1.55%.

18 Fractions were collected and evaporated to dryness in a vacuum desiccator; each fraction was quantitated on the amino-acid analyzer. α-Aminoisobutyric acid and isovaline were not separated completely on the amino-acid analyzer. Therefore, these amino acids were estimated by the areas of the peaks found on gas chromatography. The amino-acid analyzer gave the sum of threonine and allothreonine; the gas chromatography peaks, which were completely separated, indicated about equal amounts of these compounds.

The identity of the amino acids was confirmed by gas chromatography–mass spectrometry of the *N*-trifluoroacetyl-amino acid-(+)-2-butyl esters (21). All the amino acids except $\alpha,\gamma$-diaminobutyric acid were separated on a capillary column (45.7 meters) containing OV 225 as a liquid phase (we thank G. Pollock for this column). This column separated the four stereoisomers of threonine–allothreonine; the isoleucines separated into three peaks, D-alloisoleucine, L-alloisoleucine + D-isoleucine, L-isoluecine. The $\alpha,\gamma$-diaminobutyric acid was separated on a 1% OV-1 on chromosorb W column (0.6 × 180 cm). The instrument was a LKB 9000 (ionization potential, 70 V; mass range, 8–400).

## RESULTS

The chromatogram from the amino-acid analyzer is shown in Fig. 1. The peaks labeled valine, norvaline, alloisoleucine, leucine, and norleucine contain between 50 and 80% of the indicated compound. Most of the peak labeled α-hydroxy-γ-aminobutyric acid (HAB) + Ile is α-hydroxy-γ-aminobutyric acid. The *dashed line* in Fig. 1 shows the increase in color yield on heating the column eluent and ninhydrin for 30 min instead of the usual 8 min. The color yield of protein amino acids is not changed by the additional heating, but *N*-substituted amino acids, and some β-amino acids, give substantial increases in color yield (22). This is particularly noticeable in the glutamic-acid region of the chromatogram. The chromatography on Dowex 50($H^+$) cleanly separated aspartic acid, threonine, serine, and glutamic acid from interfering compounds, and allowed quantitation on the amino-acid analyzer.

Quantitation of the amino acids after elution from Dowex 50($H^+$) is given in Table 1. The identity of each amino acid

was confirmed by the gas chromatography–mass spectrometry. The elution time and the mass spectrum of an unknown and of known standards confirmed the identification based on HCl elution from Dowex 50, and elution time from an amino-acid analyzer.

In addition to confirming the identity of the unknown, the gas-chromatographic analysis showed that each of the amino acids (except for isovaline, α-hydroxy-γ-aminobutyric acid, α,γ-diaminobutyric acid, and aspartic acid that do not form two peaks on the columns used) were racemic within the experimental error (45–55% D-isomer). This result shows that there was no significant contamination from reagents or dust during the separation process. This conclusion applies particularly to the proline, where the yield was sufficiently low that contamination was a reasonable possibility. The mass spectrum of α-hydroxy-γ-aminobutyric acid is shown in Fig. 2.

## DISCUSSION

The results in Table 1 show that there is no selective synthesis of the branched-chain amino acids that occur in proteins. Indeed, the yield of norvaline is three times that of valine, although the yield of norleucine is 50% that of leucine and that of (isoleucine + alloisoleucine). Therefore, the occurrence of glycine, alanine, valine, isoleucine, and leucine in proteins, but the absence of α-amino-*n*-butyric acid, norvaline, alloisoleucine, and norleucine, cannot be understood on the basis of the yields from this type of synthesis.

The absence of *tert*-leucine in this synthesis may be due to the instability of its amino nitrile or its precursor aldehyde (pivalaldehyde). Two aliphatic amino acids with both α hydrogens substituted, α-aminoisobutyric acid and isovaline (α-amino-α-methyl-*n*-butyric acid) were found. The six-carbon amino acids of this class were not looked for. The relatively low yield of α-aminoisobutyric acid and isovaline may be due to the instability of the corresponding aminonitrile, as has been discussed elsewhere (12).

The yield of proline is quite low—seven times lower than leucine. A yield this low suggests that an electric-discharge synthesis of this type was not the only source of proline on the primitive earth.

The yield of 5- and 6-carbon amino acids is substantially lower than the glycine, alanine, or even the α-amino-*n*-butyric acid. The mole ratios of glycine:alanine:α-amino butyric acid:(valine + norvaline):6-carbon amino acids are 100:180:60:18:6. There is some variation in these ratios in different experiments, with the same conditions and spark source. The reason for the lower yields of the 5- and 6-carbon amino acids is not clear. If we lowered the temperature to 0° during the sparking, the yields of the 5- and 6-carbon amino acids were not increased, nor did the use of ethane instead of methane increase the yields.

It seems unlikely that the amino acids that were important in prebiotic polypeptides were present in the primitive ocean in about equal concentrations. Several mechanisms would have been available to concentrate certain amino acids in prebiotic polypeptides. One possible mechanism would have concentrated the sea water in a lagoon by evaporation until the amino acids were partially precipitated, and then synthesized peptides with the precipitated amino acids. The process would have concentrated the 5- and 6-carbon amino acids, since they are less soluble than glycine, alanine, and

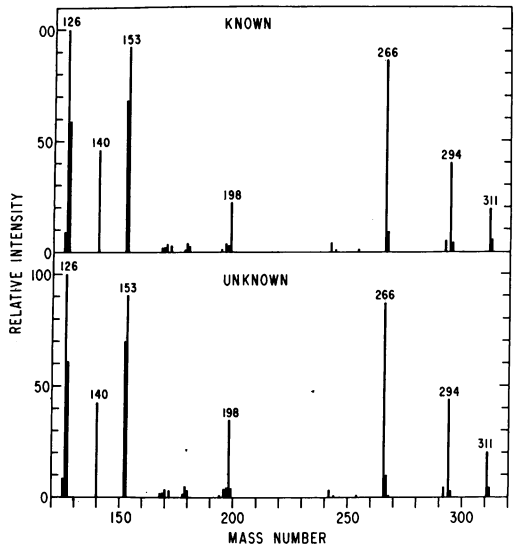


FIG. 2. The mass spectrum of the known and unknown *N,O*-ditrifluoroacetyl-2-butyl ester derivative of α-hydroxy-γ-aminobutyric acid.

α-amino-*n*-butyric acid. The concentration of the higher aliphatic amino acids could also have occurred by adsorption on suitable mineral surfaces.

Peptide bonds of the 5- and 6-carbon amino acids are more stable to hydrolysis than those of glycine and alanine. This stability has been correlated with the "rule of 6" (23), and it has been suggested that the relative rates of hydrolysis generated sequences of peptides in the primitive ocean that were hydrolytically stable (24). The same considerations would predict that the higher aliphatic amino acids would concentrate in the peptides of a primitive ocean.

These considerations make it plausible that the yields of the 5- and 6-carbon amino acids obtained in these experiments would have been adequate for prebiotic peptide synthesis.

All of the aliphatic α-amino acids reported in the Murchison Meteorite (25–28), as well as aspartic acid, glutamic acid, β-alanine, and proline, were obtained in this electric-discharge experiment. We have also found *N*-alkylated amino acids, as well as other nonprotein amino acids, in these experiments. The similarities of these amino acids with those found in the Murchison Meteorite, and their significance, will be discussed in a forthcoming paper in this journal.

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